

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1. – 33. (Cancelled)

34. (Previously Presented) A method of determining whether a tumor in a tissue sample is malignant or benign, the method comprising:

(a) selecting two wavelengths of light to minimize a difference between relative changes in intensity of light transmitted through the tumor for the two wavelengths, wherein the relative changes in intensity of the light for each wavelength are measured relative to a background intensity of light transmitted through the sample;

(b) obtaining measures of background reduced scattering coefficients of the sample at the two wavelengths;

(c) calculating an absolute oxygenation level of the tumor in the sample by using the relative changes in intensity of the light for the two wavelengths and the measures of the background reduced scattering coefficients of the sample for the two wavelengths; and

(d) determining whether the tumor is malignant or benign based on the value of the calculated absolute oxygenation level.

35. (Original) The method of claim 34, wherein the tissue sample is selected from the group consisting of breast tissue, brain tissue, and muscle tissue.

36. (Original) The method of claim 34, wherein the two wavelengths of light are in the near infrared spectrum.

37. (Original) The method of claim 34, wherein the difference between the relative changes in intensity is zero.

38. (Original) The method of claim 34, wherein the absolute oxygenation level of the tumor is calculated using a formula

$$SO_2 = \frac{\varepsilon_{Hb}(\lambda_2) - \varepsilon_{Hb}(\lambda_1) \frac{\mu'_{s0}(\lambda_1) \Delta I / I_{0|_{\max}}^{(\lambda_1)}}{\mu'_{s0}(\lambda_2) \Delta I / I_{0|_{\max}}^{(\lambda_1)}}}{[\varepsilon_{Hb}(\lambda_2) - \varepsilon_{HbO_2}(\lambda_2)] + [\varepsilon_{HbO_2}(\lambda_1) - \varepsilon_{Hb}(\lambda_1)] \frac{\mu'_{s0}(\lambda_1) \Delta I / I_{0|_{\max}}^{(\lambda_1)}}{\mu'_{s0}(\lambda_2) \Delta I / I_{0|_{\max}}^{(\lambda_1)}}}$$

wherein:

SO_2 is the oxygenation level of the region;

$\Delta I / I_{0|_{\max}}^{(\lambda_1)}$ is a maximum value of the relative change in intensity at a first wavelength in the pair of wavelengths;

$\Delta I / I_{0|_{\max}}^{(\lambda_2)}$ is a maximum value of the relative change in intensity at a second wavelength in the pair of wavelengths;

$\frac{\mu'_{s0}(\lambda_1)}{\mu'_{s0}(\lambda_2)}$ is a ratio of the background reduced scattering coefficient at the first wavelength to the background reduced scattering coefficient at the second wavelength;

$\varepsilon_{Hb}(\lambda_1)$ and $\varepsilon_{Hb}(\lambda_2)$ are molar extinction coefficients for deoxy-hemoglobin at the first and second wavelengths; and

$\varepsilon_{HbO_2}(\lambda_1)$ and $\varepsilon_{HbO_2}(\lambda_2)$ are molar extinction coefficients for oxy-hemoglobin at the first and second wavelengths.

39. (Currently Amended) The method of claim 34, further comprising, prior to selecting the two wavelengths of light:

- (e) illuminating the sample with a plurality of wavelengths of light; and
- (f) detecting light transmitted through the sample at a plurality of locations[[]],
wherein the two wavelengths of light are selected from among wavelengths of the light transmitted through the sample.

40. (Original) The method of claim 39, further comprising (g) displaying an image of the absolute oxygenation level at the plurality of locations within the tissue sample.

41. (Previously Presented) The method of claim 34, further comprising (e) storing values of the absolute oxygenation level of the tumor in a computer-readable medium.

42. (Previously Presented) A method of determining whether a tumor in a tissue sample is malignant or benign, the method comprising:

- (a) obtaining thicknesses of the sample and intensities of light transmitted through the sample at a plurality of locations for two wavelengths of light;
- (b) calculating spatial second derivatives of products of the sample thicknesses and the intensities of the transmitted light at the locations for the two wavelengths of light;
- (c) calculating an oxygenation level of the tumor based on the spatial second derivatives for the two wavelengths of light, the molar extinction coefficients of oxy-hemoglobin for the two wavelengths of light, the molar extinction coefficients of hemoglobin for the two wavelengths of light, relative changes in intensity of the light for the two wavelengths of light and the measures of the background reduced scattering coefficients of the sample for the two wavelengths of light;
- (d) calculating an oxygenation level of non-tumor regions of the tissue sample based on the spatial second derivatives for the two wavelengths of light, the molar extinction coefficients of oxy-hemoglobin for the two wavelengths of light, the molar extinction coefficients of hemoglobin for the two wavelengths of light, relative changes in intensity of the light for the two

wavelengths of light, and the measures of the background reduced scattering coefficients of the sample for the two wavelengths of light; and

(e) comparing the oxygenation level of the tumor with the oxygenation level of non-tumor regions of the sample to determine whether the tumor is malignant or benign.

43. (Original) The method of claim 42, wherein the tissue sample is selected from the group consisting of breast tissue, brain tissue, and muscle tissue.

44. (Original) The method of claim 42, wherein the two wavelengths of light are in the near infrared spectrum.

45. (Original) The method of claim 42, wherein the oxygenation level of a region of the sample is calculated using a formula

$$OL = \frac{\Delta[HbO_2]}{\Delta[HbO_2] + \Delta[Hb]}, \text{ wherein}$$

$$\Delta[HbO_2] = \frac{\left(\sum_i N''(\lambda_i) \epsilon_{HbO_2}(\lambda_i) \right) \left(\sum_i \epsilon_{Hb}^2(\lambda_i) \right) - \left(\sum_i N''(\lambda_i) \epsilon_{Hb}(\lambda_i) \right) \left(\sum_i \epsilon_{HbO_2}(\lambda_i) \epsilon_{Hb}(\lambda_i) \right)}{\left(\sum_i \epsilon_{HbO_2}^2(\lambda_i) \right) \left(\sum_i \epsilon_{Hb}^2(\lambda_i) \right) - \left(\sum_i \epsilon_{HbO_2}(\lambda_i) \epsilon_{Hb}(\lambda_i) \right)^2},$$

$$\Delta[Hb] = \frac{\left(\sum_i N''(\lambda_i) \epsilon_{Hb}(\lambda_i) \right) \left(\sum_i \epsilon_{HbO_2}^2(\lambda_i) \right) - \left(\sum_i N''(\lambda_i) \epsilon_{HbO_2}(\lambda_i) \right) \left(\sum_i \epsilon_{HbO_2}(\lambda_i) \epsilon_{Hb}(\lambda_i) \right)}{\left(\sum_i \epsilon_{HbO_2}^2(\lambda_i) \right) \left(\sum_i \epsilon_{Hb}^2(\lambda_i) \right) - \left(\sum_i \epsilon_{HbO_2}(\lambda_i) \epsilon_{Hb}(\lambda_i) \right)^2},$$

and wherein:

OL is the oxygenation level of the tumor;

i is a wavelength index for the two wavelengths;

ϵ_{HbO_2} and ϵ_{Hb} are the molar extinction coefficients of oxy-hemoglobin and deoxy-hemoglobin, respectively;

$\Delta[HbO_2]$ and $\Delta[Hb]$ are relative values for the spatial changes in the concentrations of oxy-hemoglobin and deoxy-hemoglobin, respectively; and

N'' is a spatial second derivative of an intensity of transmitted light.

46. (Currently Amended) The method of claim 42, further comprising, prior to obtaining the intensities of the transmitted light for two wavelengths:

(f) illuminating the sample with a plurality of wavelengths of light; and

(g) detecting light transmitted through the sample at a plurality of locations[.],

wherein the two wavelengths of light are selected from among wavelengths of the light transmitted through the sample.

47. (Previously Presented) The method of claim 42, further comprising (f) displaying an image of oxygenation levels at the plurality of locations within the tissue sample.

48. (Previously Presented) The method of claim 42, further comprising (f) storing values of oxygenation levels at a plurality of locations within the tissue sample in a computer-readable medium.